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No earlier studies are known concerning the process of fertilization, itself, after pollination with irradiated pollen. The cytogenetical studies of the embryo sacs after fertilization following pollen irradiation are still limited. [1–8]

No earlier cytogenetical studies in plants are known concerning the process of induction of haploid embryos or diploid and tetraploid endosperm nuclei with irradiated pollen.

The present summary surveys a series of investigations on the process of fertilization, chromosomal aberrations and other abnormalities occurring in the fertilized embryo sac, and the resulting fruit and seed formation in various species of higher plants. They represent both the binucleate and the trinucleate types of pollen. The X- and gamma ray exposures used vary from 1 to 500 kr. The present article is based on the publications listed below and referred to by Roman numerals. Some data from papers in press are also presented and discussed.

I Vassileva-Dryanovska, O. A. 1966. Fertilization in *Tradescantia.* — Hereditas *55,* 1: 47—54.

II Vassileva-Dryanovska, O. A. 1966. Tube growth following pollen irradiation in *Tradescantia.* — Comptes rendus de l'Acad, bulg sciences *19,* 7:649—651.

III Vassileva-Dryanovska, O. A. 1966. Fertilization with irradiated pollen in *Tradescantia.* — Radiation Botany.

IV Vassileva-Dryanovska, O. A. 1966. Development of embryo and endosperm produced after irradiaton of pollen in *Tradescantia.* — Hereditas 55, 2—3:129—148.

V Vassileva-Dryanovska, O. A. 1966. Induced chromosomal aberrations and other abnormalities in the embryo sac following pollen irradiation in *Melandrium rubrum.* — Hereditas 55, 2—3:149—159.

VI Vassileva-Dryanovska, O. A. 1966. The induction of haploid embryos and tetraploid endosperm nuclei with irradiated pollen in *Lilium.* — Hereditas 55, 2—3:160—165.

VII Vassileva-Dryanovska, O. A. 1966. Pollen radiosensitivity with regard to fruit and seed formation. — Comtes rendus de l'Acad. bulg. Sciences *19,* 8:759—762.

I. Fertilization

1. *Control*

The growth of the pollen tubes from the stigma to the embryo sac and process of fertilization in *Tradescantia paludosa* /I/, *Lilium speciosum* /VI/, *Melandrium rubrum* /V/, *Lycopersicum esculentum*[9], *Solanum melongena*[9] and *Ornithogalum graminifolium*[10] were described.

The process of nuclear fusion at the double fertilization in the plants of the papers /V/ [9,10] belongs to the type of *Crepis, Pisum* and other plants[11] and in *L. speciosum* to the type of *Fritillaria* /VI/. In *Tr. paludosa* a new type of fertilization, intermediate between the two types mentioned above was observed /I/.

2. *Irradiations*

The tube growth inhibition and abnormalities in the pollen tube and in the fertilization, in the plants mentioned above /II, III, V, VI/, [9,10] increase depending on the exposures.

Depending on the abnormalities in the male chromatin the process of fertilization is realized in two different ways namly: a/ double fertilization b/ single fertilization of only the one or the other of the two types of female nuclei.

With respect to the nuclear fusion the following types were observed:

A. In *Tr. paludosa* /III/:

(a) A normal fusion as in untreated material (1—500 kR).

(b) The nuclear fusion occurs but the normal changes in chormatin structure of the female nuclei do not occur (50—500 kR). Most of these nuclei do not divide (50—500 kR), but a few may be stimulated to start division. (150—500 kR).

(c) Nuclear fusion does not occur but the male chromatin is appressed to the female nucleus (50—500 kR), which may stimulated to start division (150—500 kR).

B. In *L. speciosum* /VI/:

(a) In the egg nucleus and in the polar nuclei a normal fusion occurs as in control material. The diploid zygote and the pentaploid endosperm start to divide with elimination of fragments into the cytoplasm (1—20 kR).

(b) The polar nuclei are induced to fuse with one another by the pycnotic male chromatin appressed to one of them (50—500 kR). At premetaphase, of the first division, the male chromatin contracts to a dense mass. During mitoses only the female chromosomes are arranged in the metaphase plate. The male chromatin may stick to some of the female chromosomes, or may remain in the cytoplasm.

C. In *M. rubrum* /V/:

(a) In the egg nucleus and in the central nucleus a normal fusion occurs as in control material (1—500 kR).

(b) In the egg nucleus after a normal fusion and certain structural transformations of the sperm chromatin the region containing the male chromatin takes a rounded shape and gives the impression of being a separate smaller nucleus lying within the egg nucleus. This kind of zygote nucleus does not divide (10—500 kR).

(c) In the egg nucleus the sperm chromatin remains on the surface and later forms a special region having the appearance of a Feulgen negative nucleus appressed to the female nucleus. This kind of zygote nucleus does not divide (50—500 kR).

D. In the other studied plants the fusion occurs as in control material (*1—500 kR*) [9,10].

II. Embryogeny

1. *Control*

In all studied plants the development of the embryo and endosperm take place in normal way without bridges or elimination of fragments into the cytoplasm /II, V, VI/. [9,10].

2. *Irradiations*

The development of the embryo and endosperm in all studied plants takes place in different ways, depending on the exposures. In the embryo, at all the used exposures, only acentric fragments /*Tr. paludosa, L. speciosum*/ micronuclei /*L. speciosum*/ and lobed nuclei /*Tr. paludosa, L. speciosum, M. rubrum, O. graminifolium*/ were found. Bridges were not observed. Degeneration starts earlier in the embryo and is more pronounced than in the endosperm. In *Tr. paludosa* /IV/ some cases of induction of haploid embryos by picnotic male chromatin irradiated with higher exposures were obtained. In *L. speciosum* /VI/ at higher exposures some cases of haploid metaphases and pseudogamy in the egg cell were found.

The following different types of development were obtained in the endosperm:

(A) After a normal fusion of the male chromatin with the polar nuclei, /IV, VI/ or with the central nucleus /V/ [9, 10] as in untreated material.

(a) Normal mitosis.

(b) Abnormal mitosis.

(B) After stimulation of the polar nuclei to fuse by the pycnotic male chromatin. Diploid /*Tr. paludosa*/ and tetraploid /*L. speciosum*/ prophases, metaphases and anaphases of the first division of the endosperm were found /IV, VI/. In *L. speciosum* the endosperm is later developed as (a) abnormal, (b) normal (most of the nuclei) and abnormal (only some of the nuclei) in the same embryo sac, or (c) normal.

In the endosperm the following types of chromosomal aberrations were found:

(1) Single or multiple dicentric bridges with acentric fragments, with different fraquency depending on the plants and exposures. At the higher exposures the frequency of multiple bridges increase /IV, V, VI/ [9, 10].

(2) Sticky aberrations with partial elimination of the male or female chromatin into the cytoplasm and such aberrations without elimination at higher exposures /IV, V, VI/ [9, 10].

(3) Some instances of partial or full elimination of the male chromatin into the cytoplasm were observed at exposures 5 and 10 kR, with the formation of approximately diploid or aneuploid metaphases /IV/.

With respect to the fruit and seed formation some results were presented /IV, V, VII/.

III. Discussion

Some of the results and conclusions and their significance are discussed briefly below. It was obtained modifying effects of the radiation, especially of the higher exposures on the process of fertilization. These effects might open some interesting experimental potentialities.

In plants [5, 14, 24–27] and in animals [28–32] haploidy induced by irradiation has been studied. From the present study it appears that there may be three different ways of formation of haploid embryos or haploid, diploid or tetraploid endosperm nuclei of maternal origin after pollination with irradiated pollen:

(a) After fertilization of the female nucleus by destroyed male chromatin which in the first divisions may be eliminated into the cytoplasm. /*Tr. paludosa*/.

(b) After a stimulation of the female nuclei to divide by the pycnotic male chromatin /*Tr. paludosa, L. speciosum*/.

(c) After a stimulation of the egg nucleus to divide without fertilization (pseudogamically) under influence of the developing endosperm /*L. speciosum*/.

Further studies in higher plants of induced stimulated development of female nuclei by pollen irradiated with higher doses my be directed to the purpose of receiving vital haploidy /diploidy when starting from tetraploid/.

The cytologically visible lethal effects and reduction of the fruits and seeds were found to be dose dependent processes. The degeneration is more pronounced in the embryo than in the endosperm [1, 20, 21].

The author suggested that this difference between embryo and endosperm might be due to differences in metabolic processes between these two tissues as well as such factors as differences in nuclear size and especially in their number of chromosomes. Some authors [7, 33—37] have demonstrated lethality due to induced dominant lethals in the male gametes. As appears from the present study among the factors and the cytological abnormalities responsible for dominant lethality of the zygote, embryo and endosperm the following may be of importance:

(a) Induced dominant lethals in the male chromatin.
(b) Unfertilization, incorporations without divisions, or nonfusion.
(c) Initial toxic effects.
(d) Genetic unbalance.

At all the used exposures and species a lack of bridges was found in the zygotes and embryos. Nor the breakage-fusion-bridge cycle was found in the embryo cells [5, 39, 40]. The main reason that no bridges occur between the daughter[38] nuclei in the embryos is probably that a cell wall is formed between these nuclei. There might also be a stronger tendency for open breaks to heal in the zygote instead of recombining to give dicentrics and other structural changes. This factor should reduce the frequency of bridges but not eliminate them. Besides the factors mentioned various other effects could be involved such as differences between zygote and endosperm in metabolic processes or other physiological conditions.

The idea /IV, V/ concerning the possibility that irradiated male chromatin may induce or provoke breaks in the female chromatin raises some questions concerning the ways in which an effect of this kind might originate. This effect might be connected with the well-known ability of open breaks to undergo union, and also with the observed stickiness of the strongly irradiated male chromatin that makes it stick to the female one. Some authors[41—45] have reported that some radiation breaks may remain open for about 1—2 hours. Others[45—47] have pointed out the importance of enzymes for the reunion of open breaks and of metabolism in modifying nuclear demage. It should be mentioned that high exposures of irradiation are capable of depressing the activity of enzymes[48].

It may be supposed that some of the breaks induced in the irradiated pollen may remain open for a longer time or even undergo temporary healing and through these processes the breaks might be available for later unions during and after fertilization. Some of these open breaks might get attached to the female chromatin to cause stickiness or induce breaks in it. An increased exposure of irradiation will, in itself, cause an increased frequency of breaks, but with the increased exposure another effect acting in a similar

manner may also become very important, namely a radiation-induced inactivation of enzymes necessary for the reunion of the breaks. Consequently, the strong irradiations might cause an enormous increase in the number of breaks that remain open.

The effect of the irradiated pollen on the fruit and seed formation has been investigated [6, 12–23, 51].

The importance of chromosome size and chromosome number for the radioresistance has been demonstrated in studies on the somatic development [49, 50] As appears from the present study among the factors possibly responsible for the difference in radioresistence of the pollen grains the following may be of importance: a) the type of the pollen grains (trinucleate versus binucleate) b) the stage in the nuclear cycle of the male chromatin c) the number of the chromosomes d) the size of the chromosomes e) the presence or absence of a pollen tube mitosis after irradiation.

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